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Evaluation of the Production of Biosurfactant by Yeast Strains Isolated from Fruit Pastes and their Biodegradative Potential on Waste Engine Oil

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Abstract

Oil pollution and remediation technology have become a global phenomenon of increasing importance. Most of the hydrocarbons are insoluble in water and their degradation using microorganisms has an important role in combating environmental pollution. This study was undertaken to evaluate the production of biosurfactant by a yeast strain isolated from fruit pastes and its biodegradative potential on waste engine oil. Yeast strains were isolated from ripe pineapple (*Ananas comosus*) and unripe plantain (*Musa paradisiaca*) pastes and screening for their biosurfactant potentials using emulsification test. Gravimetric method using N – hexane as extractive solvent was employed in determining the biodegradative potentials of the yeast consortium and their biosurfactants on waste engine oil contaminated soil and seawater, respectively. The result revealed that a total of 10 strains of yeast were isolated from the fruit pastes. The yeast strain PA4 had the highest emulsification activity of 37.50 % while yeast strain PA1 had the lowest emulsification activity of 23.52 %, respectively. The lowest percentage oil removal values of 22.0, 43.8, 57.2 and 79.4 % were obtained at day 1 while the highest percentage oil removal values of 97.4, 94.0, 96.0 and 96.2 % were obtained at day 60 in all the polluted soil treatments, respectively with non - significant differences ($p > 0.05$) detected among the means of different treatment conditions and remediation period in comparison to the control setup. Also, the lowest percentage oil removal values of 11.2, 20.40, 52.8 and 73.2 % were obtained at day 1 while the highest percentage oil removal values of 84 .0, 85.8, 80.2 and 84.0 % were obtained at day 30 in all the polluted seawater treatments, respectively with significant differences ($p < 0.05$) detected among the means of different treatment conditions and remediation period in comparison to the control setup. The biodegradation data fit well into pseudo first order kinetic model and value of the kinetic parameter showed that the degree of effectiveness of these bioremediation strategies in the cleanup of soil contaminated with waste engine oil is in the following order: C3 < C2 < C1 < Co while the cleanup of seawater contaminated with waste engine oil is in the following order: Co < C3 < C2 < C1, respectively. Thus, yeast consortium and the biosurfactants produced, besides being obtained from low cost substrates, revealed effectiveness in the clean-up and dispersion of waste engine oils in soil and seawater, allowing the replacement of physical and chemical treatment agents with environmental friendly agents. The study has also helped to bridge the gap of knowledge on isolation of biosurfactant producing yeast strains from non – polluted sources.

Keywords: Bioaugmentation, Biosurfactant, Half - life, Kinetics, Yeast.

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1. Introduction

An increasing need for materials, energy, and water forces the shift from a fossil-based linear economy to a sustainable circular bioeconomy. With the bioeconomy concept high on the global agenda, search for biological feedstock that has the potential to generate a spectrum of bio-based products has been initiated in many areas of research and industry (Enemchukwu *et al*., 2026a; 2026b). In this context, biogenic waste is considered as a potential feedstock for developing a circular bioeconomy. Surfactants are among the most important bulk chemicals that are used in almost every product and activity of human daily life, such as cleaning products, cosmetics, food, textiles, pharmaceuticals, mining, agriculture, paper production, etc. Biosurfactants are an alternative to synthetic surfactants synthesized from fossil resources (Jiménez-Peñalver *et al*., 2019). BSs are structurally diverse amphiphilic molecules produced by a variety of microorganisms— fungi (e.g. yeasts) and bacteria, especially *actinomycetes* (De *et al*., 2015). Microorganisms synthesize BSs as secondary metabolites that can either remain attached to the cell surface or be secreted outside the cell (Jahan *et al*., 2020). Some yeast species, such as *Starmerella bombicola* and *Pseudozyma antarctica*, are proven to be exceptional BS producers (Jezierska *et al*., 2017). In many aspects, yeasts are superior to bacterial producers. Yeasts do not produce toxic byproducts; many yeast species are used for making traditional food and beverages. Pathogenic strains are seldom (if any) among the yeast species used for biotechnological processes (Campos-Takaki *et al*., 2010). Yeasts can produce BS from lipid-rich substrates: vegetable oils, fats, and their products. BSs have several advantages over their synthetic equivalents: (1) BSs can be produced from renewable feedstocks by fermentation; (2) Biosurfactants have greater environmental compatibility, as they are readily biodegradable and display low toxicity; and (3) Biosurfactants show better foaming properties and stable activity at a wide range of conditions (pH, salinity, and temperature) (Saharan *et al*., 2012).

Although biosurfactants have many advantages over their synthetic analog, biosurfactants are not yet competitive with synthetic surfactants due to very high production costs resulting from significantly low BS yields in the fermentative processes (Dhanarajan and Sen, 2014). Still in 2025, the global BSs' market is expected to be around 4.8 billion euro at a compound annual growth rate of 5.5% in the forecast period 2020–2025 (https://www.marketresearchengine.com/biosurfactants-market, 2020). So for bioproducts to gain their market share, the quality and price must be comparable to their synthetic analogs (Mukherjee *et al*., 2006; Nitschke *et al*., 2005). Biosurfactants can replace synthetic surfactants only if the cost of the raw material and the process is reduced to the minimum (Sobrinho *et al*., 2008). Exploring alternative substrates for biosurfactant production can serve as an effective cost-cutting strategy. The use of low-cost substrate or cheap waste material may decrease the total biosurfactant production costs by 10 to 30% (Zenati *et al*., 2018).

Petroleum exploration, exploitation, refining, transportation and storage in the Niger Delta and other neighboring states in Nigeria constitutes dangers to the public health and environment. These increasing environmental challenges attract interests on research for alternative biosurfactants as chemical and other synthetic surfactants are non-biodegradable and toxic to both aquatic and terrestrial bioindicators. Most biosurfactants production especially from bacteria results in low surfactant quantities and are expensive due to high cost of substrates. Most of the sources of biosurfactant producing microorganisms do not exclude the use of pathogenic organisms. Various studies on biosurfactants were dominated by bacteria and moulds with few reports on yeasts. Yeasts are generally regarded as safe and have the ability to produce higher quantities of surfactants than bacteria. Therefore, the study was undertaken to evaluate the production of biosurfactant by a yeast strain isolated from fruit pastes and its biodegradative potential on waste engine oil.

2. Materials and Methods

2.1 Sample Collection

Ripe Queen Pineapple (*Ananas comosus*) and Plantain (*Musa paradisiaca*) fruits were purchased from Ekeagbagba Market, Uli town in Ihiala Local Government Area (LGA) Anambra State Nigeria. The fruits were carried in a sterile plastic container, identified by a botanist in Biological Sciences, Chukwuemeka Odumegwu Ojukwu University and transported to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University Uli Campus for further analyses.

2.2 Sample Preparation

The fruits were washed with sterile water containing disinfectant, drained, peeled and sliced into smaller pieces. The juices were aseptically extracted using an electric juice blender, filtered with muslin cloth and the filtrates were collected in a sterile plastic container as described by Anichebe *et al.* (2019); Okoye *et al.* (2020a); (2020b); (2020c) and Uba *et al.* (2020a).

2.3 Preparation and Sterilization of Media and Glassware

All glasswares used were sterilized in an autoclave at 121 ℃ at 15 psi for 15 min. Yeast Extract Dextrose Peptone (YEDP) broth was used for enrichment and isolation while Potato Dextrose Agar (PDA) was used for the inoculation and identification of the yeast isolates. Yeast Extract Dextrose Peptone (YEDP) broth was prepared with 40 g of peptone water, 10 g of yeast extract and 20 g of dextrose sugar (sucrose) in 1 L of distilled water. The mixtures were mixed vigorously and then sterilized by autoclaving at 121 ℃, 15 psi for 15 min. It was allowed to cool before inoculation. Fifty (50) mg/L of tetracycline and 0.05 mg/L of gentamycin were added to inhibit bacterial growth. Also, Potato Dextrose Agar (PDA) medium was used for the isolation of the yeast. This was prepared according to manufacturer's instructions by dispensing 39 g of agar into 1000 mL of distilled water. The mixture was mixed vigorously and then sterilized by autoclaving at 121 ℃, 15 psi for 15 min. It was allowed to cool to 45 ℃ and dispensed into Petri dishes for solidification and dried in a hot air oven before inoculation. Fifty (50) mg/L of tetracycline and 0.05 mg/L of gentamycin were added to the PDA media to inhibit bacterial growth (Dibua *et al*., 2020; Chukwura *et al*., 2025).

2.4 Isolation and Maintenance of the Pure Yeast Culture

For the isolation of hydrocarbon - degrading yeasts, 2 % (v/v) of waste engine oil was added into a 250 mL conical flask containing 100 mL of YEDP broth with 100 mL of the fermenting extracts of plantain and pineapple, respectively were incubated for two weeks at 30 ℃ to enhance microbial growth. An aliquot of 0.5 mL of the YEDP broth containing the plantain and pineapple substrates juices were inoculated onto Potato Dextrose Agar (PDA) media in duplicates using a glass spreader. The plates were incubated at 30 ℃ for 72 hr. The colonies that appeared on the plates were further sub - cultured and incubated for another 48 hr at 30 ℃ in order to obtain pure cultures. The selected yeast isolates were obtained and the pure cultures of the isolates were stored in 10 % glycerol at 4℃ in Bjou bottles (Nwaguma *et al.,* 2016; Uba, 2018a; 2018b; Uba *et al*., 2018a; 2018b; 2018c).

2.5 Substrate

Three types of industrial waste were used as substrates to produce the biosurfactant. Cassava liquor was obtained from a local cassava processing firm at Aluora Uli, Ihiala Local Government Area (LGA) Anambra State Nigeria. Corn steep liquor was acquired from local pap dealer at Mgbekei Uli and vegetable waste frying oil was obtained from a cake fryer at Ubahudara Uli, both Ihiala L.G.A. Anambra State, Nigeria.

2.6 Static Growth Condition

The biosurfactant production conditions that was used in this work was adopted with slight modification from the method of Da Silva Lira *et al.* (2020). The inoculum of yeast was prepared by transferring cells grown on a slant with 50 mL of yeast mold broth (YMB). The seed culture was incubated for 24 hr at 28 °C and agitated at 200 rpm. The yeast was cultivated in a submerged culture with shaking in a rotary shaker. A low-cost basal production medium formulated with distilled water supplemented with 5 % waste vegetable oil, 5 % cassava liquor and 5 % corn steep liquor were used for production of the biosurfactant. The medium was sterilized by autoclaving at 121 °C for 20 min. The final pH of the medium was adjusted to 7.0. The inoculum (5 % v/v) was added to the cool medium at the amount of 10^8 cells/mL. Cultivation was carried out in Erlenmeyer flasks at 30 °C with shaking at 180 rpm for 120 hr. After the incubation period, the production media were subjected to centrifugation with stirring at 4,000 rpm for 20 minutes for the obtainment of the cell **-** free broth. Aliquots were withdrawn after fermentation and screened for extracellular biosurfactant production. All experiments were conducted in triplicate (Uba *et al.* 2017; Dokubo *et al*., 2022a; 2022b; Dokubo and Uba, 2023; Ekwenze *et al*. 2025; Okafor *et al*. 2023; Ele *et al*. 2024a; Ifediegwu *et al*. 2024a; Uba and Okonkwo, 2025; Uba *et al*., 2025).

2.6.1 Emulsification index

The emulsifying capacity was evaluated by emulsification index (E_{24}) as described by Kalyani *et al.* (2014), Uba *et al.* (2018d), Anidu *et al*. (2023) and Uba and Anidu (2023). For this assay, 2 mL of waste engine oil was dispensed into a test tube which comprised of 2 mL biosurfactant solution. The test tube was maintained for 24 hr after being vigorously vortexed at 4000 rpm for 2 min and the emulsion height, oil and aqueous zones were measured. The emulsion index (E_{24}) was determined as the percentage of height of the emulsified layer (mm) divided by the total height of the liquid column (mm). The percentage of emulsification index was calculated using the following equation:

$$E_{24} = \frac{\text{Height of emulsion formed}}{\text{Total height of solution}} \times \frac{100}{1}$$

2.7 Characterization and Identification of the Most Biosurfactant – Producing Yeast Isolate

The best selected biosurfactant – producing yeast isolate with maximum surfactant activities was examined microscopically. Water mount was employed and sterile distilled water was placed on a glass slide with a bacteriological loop. A light emulsion of the yeast in this drop of water was made, covered with a cover slip and examined under X40 objective lens for cellular characteristics, ascospore formation and vegetative reproduction. Biochemical features examined included carbohydrates fermentation test (glucose, galactose, sucrose, maltose, fructose, lactose, xylose, arabinose), germ tube, pellicle formation and urease test (Nwaguma *et al.* 2016; Uba *et al*. 2019a; Dokubo *et al*., 2024).

2.8 Evaluation of Biodegradation Capacity of Oil Adhered to Sand

In this study, samples of 50 g of sand contaminated with 10 % waste engine oil was added to 245 mL of distilled water in a ½ L conical flask and the mixture was enriched with 5 mL of cassava liquor acquired from a local cassava plant. The mixture was sterilized by autoclaving and solutions of the crude biosurfactant at 100%, 50% and 7.5 mL of yeast previously cultured in its preparation medium (YMB) was added as presented in Table 1 below. The mixtures were incubated at 150 rpm for 90 days at 30 ºC. One percent molasses was added to the mixtures every 15 days of the experiment five times (15, 30, 45, 60 and 75 days). Samples (100mL) were removed every 15 days (1, 15, 30, 45, 60, 75 and 90 days) for the determination of the percentage of motor oil removed from the sand totaling seven samples. The percentage of oil removal was calculated by the amount of oil removed by hexane and HCl extraction technique and determined by gravimetry by adopting the modified methods of Egurefa *et al*. (2020a); (2020b); Nnaka *et al*. (2024); Uba and Chukwura, (2016); Uba (2019a); (2019b); (2019c); Uba *et al*. (2019b); (2019c); (2019d); Uba *et al*. (2020a); Da Silva Santos *et al*. (2020), Ifediegwu *et al*. (2023a) and Uba and Obiefuna (2023).

Table 1: Study design for sand washing waste engine oil

Mixture	Composition
Control	Contaminated sand + cassava liquor
Condition 1	Contaminated sand + cassava liquor + yeast isolate
Condition 2	Contaminated sand + cassava liquor + biosurfactant from yeast isolate at 50 % dilution
Condition 3	Contaminated and + cassava liquor + biosurfactant from yeast isolate at 100 % dilution

2.9 Kinetic Model Analysis

The biodegradation rate of organic compounds by microorganisms is often described by the equation as follows (Agarry and Oghenejoboh, 2015; Uba *et al*., 2019e; Ubani *et al*., 2025):

$$q = \frac{qmC}{k + C} \text{ ------------------- Eq. 3}$$

Where q is biodegradation rate, qm is maximum specific biodegradation rate, c is the substrate concentration and k is half-saturation constant. If c « k; Eq. (3) can be reduced to:

$$q = \frac{qmC}{k} \text{ ------------------- Eq. 4}$$

Eq. (4) is a typical first-order model. The use of first order kinetics in the description of biodegradation rates in environmental fate models is common because mathematically the expression can be easily incorporated into the model (Agarry and Oghenejoboh, 2015; Uba *et al*., 2019e; Ubani *et al*., 2025). Assuming k_1 = (qm/k) and integrating Eq. (4), the following relation of substrate concentration to time can be obtained as given in Eq. (5):

$$\ln c = a + k_1 t \text{ ----------------Eq. 5}$$

2.10 Estimation of Biodegradation Half-Life Time

Biodegradation half-life times ($t_{1/2}$) were calculated using Eq. (6) (Agarry and Oghenejoboh, 2015; Uba *et al*., 2019e; Ubani *et al*., 2025):

$$t_{1/2} = \frac{\ln 2}{k} \quad \text{.................Eq. 6}$$

Where k is the biodegradation rate constant (day^{-1}). The half-life model is based on the assumption that the biodegradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in soil and water.

2.11 Statistical Analysis

Data was presented as mean and standard deviation as well as analyzed using GraphPad Prism Version 8.0.2. Two-way analysis of variance (ANOVA) followed by Dunnett comparison of means of the different biodegradation and bioremediation setups at 95 % confidence level. Threshold values < 0.05 were considered statistically significant (Ifediegwu *et al*. 2023b; 2024b; 2024c; Ofunwa *et al*. 2024; Okoye *et al.*, 2020d; Njoku *et al*., 2019a; 2019b; Nkamigbo *et al*. 2020a; 2020b; Uba *et al*., 2020b; 2020b; Okafor et al. 2021a; 2021b; Alfred *et al*., 2023; Ubani *et al.*, 2024; Alfred *et al*. 2025).

3. Results

The result of the emulsification of the biosurfactant producing yeast isolate is presented in Table 2. From the result, yeast strain PA4 had the highest emulsification activity of 37.50 % while yeast strain PA1 had the lowest emulsification activity of 23.52 %, respectively.

Table 2: Emulsification activity of the biosurfactant producing yeast isolate

Isolate code	Emulsifying activity (E_{24}) (%)
PA1	23.52 ± 0.009
PA2	33.33
PA3	26.66
PA4	37.50
PA5	33.33
PL1	25.00
PL2	25.00
PL3	26.66
PL4	26.31
PL5	33.33

The result of the microscopic and biochemical feature of the best selected biosurfactant producing yeast isolate is presented in Table 3. From the result, both isolates PA3 and PA4 were rod in cell characteristics, positive to Gram stain, ascospore formation, vegetative reproduction, lactophenol stain, pellicle formation, urease, galactose, glucose, fructose, arabinose tests and negative to xylose, maltose, lactose, sucrose tests, respectively. They are physiologically identified as *Trichosporon* sp. and *Candida* sp.

Table 3: Microscopic and biochemical feature of the best selected biosurfactant producing yeast isolate

Parameters	PA3	PA4
Cell characteristics	Rod	Rod
Gram stain	+	+
Ascospore formation	+	+
Vegetative reproduction	+	+
Lactophenol stain	+	+
Pellicle formation	+	+
Urease test	+	+
Galactose	+	+
Glucose	+	+
Xylose	-	-
Maltose	-	-
Fructose	+	+
Arabinose	+	-
Lactose	-	-
Sucrose	-	-
Identity	*Trichosporon* sp.	*Candida* sp.

The result of the weight of oil recovered during 60 days of degradation of engine oil contaminated soil is shown in Figure 1. From the result, highest values of 3.90, 2.81, 2.14 and 1.03 g were recovered at day 1 while lowest values of 0.13, 0.30, 0.20 and 0.19 g were recovered at 60 days in all the treatments, respectively. The result of the weight of oil recovered during 30 days of degradation of engine oil contaminated seawater is shown in Figure 2. From the result, highest percentage oil removal values of 4.44, 3.98, 2.36 and 1.34 g were recovered at day 1 while lowest percentage oil removal values of 0.80, 0.71, 0.99 and 0.80 g were recovered at 30 days in all the treatments, respectively. The result of the percentage of oil removed from the engine oil contaminated soil after 60-day incubation period is illustrated in Figure 3. From the result, the lowest percentage oil removal values of 22.0, 43.8,57.2 and 79.4 % were obtained at day 1 while the highest percentage oil removal values of 97.4, 94.0, 96.0 and 96.2 % were obtained at day 60 in all the treatments, respectively. The result of the percentage of oil removed from the engine oil contaminated seawater after 30-day incubation period is illustrated in Figure 4. From the result, the values of 11.2, 20.40, 52.8 and 73.2 % were obtained at day 1 while the values of 84.0, 85.8, 80.2 and 84.0 % were obtained at day 30 in all the treatments, respectively.

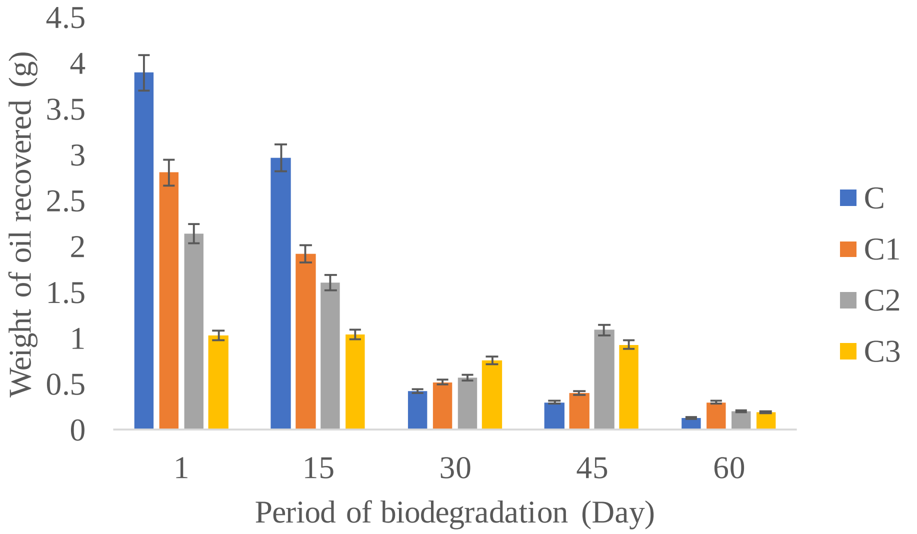

Figure 1: Weight of oil recovered during 60 days of degradation of engine oil contaminated soil

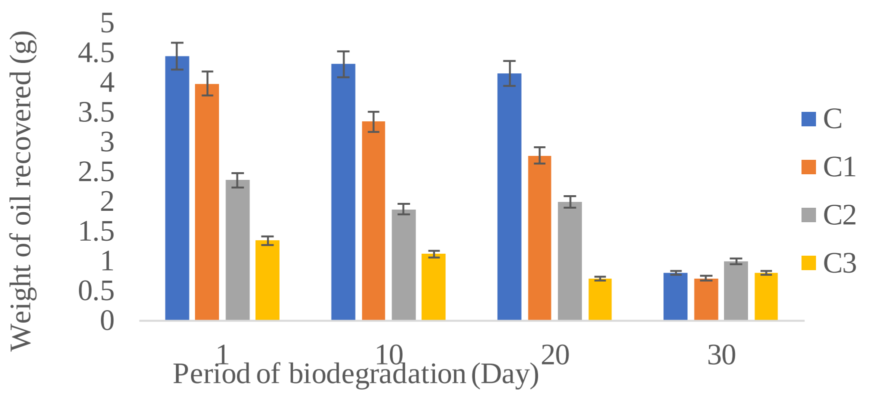

Figure 2: Weight of oil recovered during 30 days of degradation of engine oil contaminated seawater

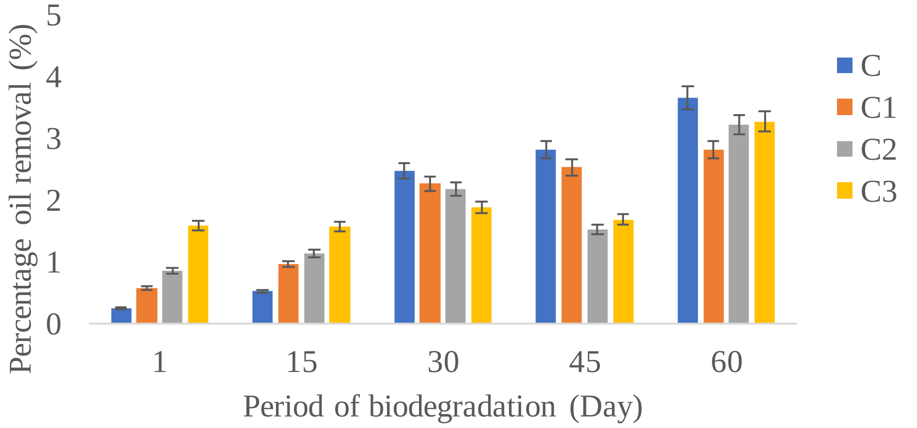

Figure 3: Percentage of oil removed from the engine oil contaminated soil after 60-day incubation period

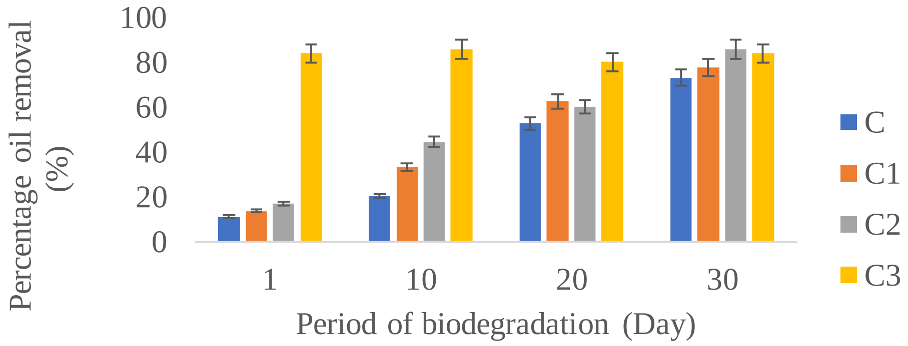

Figure 4: Percentage of oil removed from the engine oil contaminated seawater after 30-day incubation period

The result of the first-order kinetic model equation fitted to the waste engine oil contaminated soil biodegradation subjected to yeast and surfactant treatments is illustrated in Figure 5. From the result, the rate constants (k) of the consortium and their biosurfactants during the waste engine oil contaminated soil biodegradation process ranged from ≥ 0.248 to ≤ 0.060, ≥ 0.576 to ≤ 0.046, ≥ 0.848 to ≤ 0.053 and ≥ 1.579 to ≤ 0.054 day^{-1} for control, consortium, 50 % consortium and biosurfactant as well as 100 % consortium and biosurfactant treatment setups during the study after 60 days' biodegradation period, respectively.

The result of the first-order kinetic model equation fitted to the waste engine oil contaminated seawater biodegradation subjected to yeast and surfactant treatment is illustrated in Figure 6. From the result, the rate constants (k) of the consortium and their biosurfactants during the waste engine oil contaminated seawater biodegradation process ranged from ≥ 0.118 to ≤ 0.061, ≥ 0.228 to ≤ 0.065, ≥ 0.750 to ≤ 0.053 and ≥ 1.316 to ≤ 0.061 day^{-1} for control, consortium, 50 % consortium and biosurfactant as well as 100 % consortium and biosurfactant treatment setups during the study after 30 days' biodegradation period, respectively.

The result of half-life times and reaction kinetics of the biodegradation of oil contaminated soil and seawater by the consortium of yeast isolates and their biosurfactant is shown in Table 4. From the result, the highest half-life ($T^{1/2}$) times were 15.0700 and 13.0800 days, while the lowest half-life ($T^{1/2}$) times were 11.5500 and 10.6600 days during the biodegradation process of the waste engine oil in contaminated soil and seawater, respectively. Linear regression analysis was used to generate the correlation coefficient (R^2) values shown in Table 4 and obtained from the linear plots depicted in Figures 5 - 6. The high correlation coefficient (R^2) values of between 0.85 and 1 indicated a good fit of the biodegradation data to the first-order kinetic model. The results of the study revealed that there was no statistically significant difference ($p > 0.5$) between the biodegradation treatment set ups.

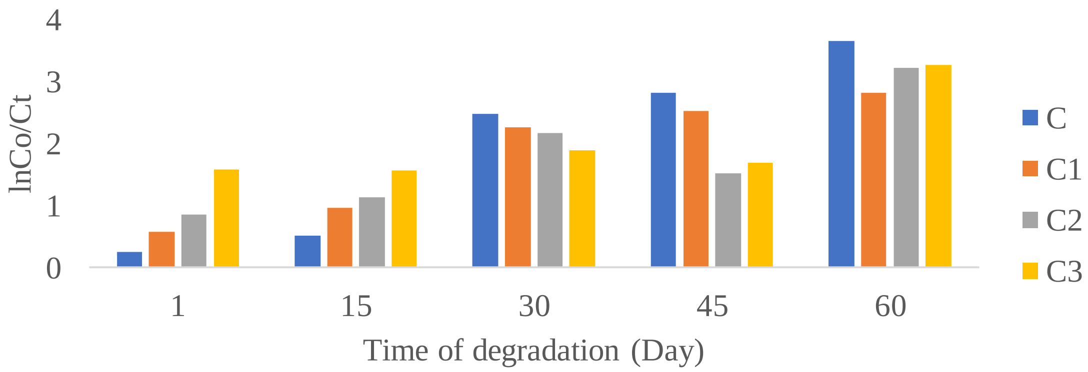

Figure 5: First-order kinetic model equation fitted to the waste engine oil contaminated soil biodegradation subjected to yeast and surfactant after 60 days of treatment

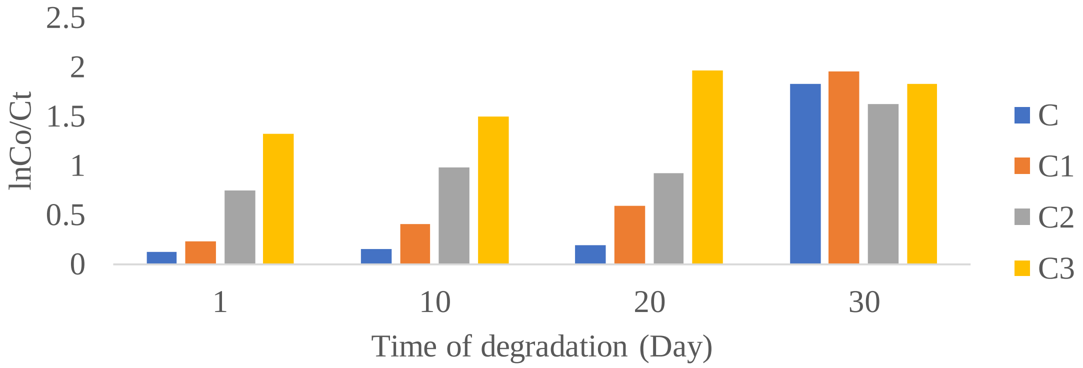

Figure 6: First-order kinetic model equation fitted to the waste engine oil contaminated seawater biodegradation subjected to yeast and surfactant after 30 days of treatment

Table 4: Half-life times and reaction kinetics of the biodegradation of oil contaminated soil and seawater by the consortium of yeast isolates and their biosurfactant

Bioremediation set up	Equation	K	R^2	$T^{1/2}$
Contaminated soil				
C_0	Y = 0.0615X + 0.0839	0.0615	0.9363	11.5500
C_1	Y = 0.0409X + 0.5937	0.0409	0.9190	15.0700
C_2	Y = 0.0347X + 0.7307	0.0347	0.7381	13.0800
C_3	Y = 0.0237X + 1.2801	0.0237	0.5904	12.8300
Contaminated seawater				
C_0	Y = 0.0541X - 0.2534	0.0541	0.6481	11.3600
C_1	Y = 0.0557X - 0.0562	0.0557	0.7876	10.6600
C_2	Y = 0.0264X + 0.6663	0.0264	0.7542	13.0800
C_3	Y = 0.0207X + 1.3365	0.0207	0.7534	11.3600

4. Discussion

Bioremediation played an important role in the cleaning of the spillage of 41 million liters of oil by the oil tanker Exxon Valdez in the Gulf of Alaska in 1989, giving rise to the development of this technology and demonstrating that there are good reasons to believe in the effective application of this method for the treatment of future oil spills under appropriate circumstances. While it was difficult to evaluate the effects of treatment due to the heterogeneity of the contamination, other studies have demonstrated the importance of the use of surfactants to enhance the biodegradation of oil (Luna *et al*., 2018). In this study, the production of biosurfactant by a yeast strain isolated from fruit pastes and its biodegradative potential on waste engine oil was evaluated. A total of 10 strains of yeasts were isolated from ripe pineapple (*Ananas comosus*) and unripe plantain (*Musa paradisiaca*) pastes. All these isolates were initially screened for extracellular bio-surfactant production grown in Yeast Extract Dextrose Peptone medium containing waste engine oil as sole source of carbon. Out of these 10, only 2 isolates (PA3 and PA4) showed higher positive result for emulsification test. As per the results observed for these two isolates, the emulsified layer for PA4 showed maximum emulsification area of 37.50 % indicating a good biosurfactant production. This result is presented in the Table 2. The use of these techniques is similar to the report of Ghayyomi *et al.* (2012) who used the combination of emulsification index and surface tension test to select biosurfactant producers. Ghayyomi *et al.* (2012) suggested that a single method is not suitable to identify biosurfactants producers, and he recommended the combination of methods.

The results in Figures 1 and 3 revealed that the addition of the yeast consortium and biosurfactants increased the oil removal rate comparable to the condition without consortium and biosurfactant. Although the highest removal rates (97.4 %) were achieved at the 60-day evaluation with the condition without consortium and biosurfactant, the conditions with 50 and 100 % biosurfactants (C2 and C3) had greater removal rates (96.2 and 96.0 %) than condition yeast consortium only (94.0 %). Statistically, there were non - significant differences ($p > 0.05$) detected among the means of different treatment conditions and remediation period in comparison to the control set up. Thus, the concentration of the isolated biosurfactant exerted an influence on the removal rate, with an increase in the mobilization and subsequent solubilization of the waste engine oil in the aqueous phase by the biosurfactant. In a study conducted by Chaprão *et al.* (2015), biosurfactants from *Candida sphaerica* and *Bacillus* sp. achieved oil removal rates of 70 and 80 %, respectively. Moreover, Santos *et al.* (2017) demonstrated the considerable capacity of a biosurfactant produced by *C. lipolytica* regarding the removal of motor oil and petroleum adsorbed to sand. Using a biosurfactant produced by *P. cepacian*, Soares da Silva *et al.* (2017) found removal rates greater than 70 %, with maximum removal (96 %) achieved when the isolated biosurfactant was used at a concentration of 2 × CMC which uphold the observation made in this study. Therefore, the bioremediation technique in waste engine oil contaminated soils demonstrates a positive role of biosurfactants in pollutant biodegradation (Mao *et al.*, 2015).

The results in Figures 2 and 4 revealed that the best result was achieved at 30 days, with 85.8 % oil removal with condition of yeast consortium only (C1) followed by 84.0 % oil removal with the condition of 100 % biosurfactant (C3). However, lower concentrations of biosurfactant (Condition 2 with 50 %) also achieved good results (80.2 %), with the removal rate increasing over time. Statistically, there were significant differences ($p < 0.05$) detected among the means of different treatment conditions and remediation period in comparison to the control set up. Santos *et al*. (2017) reported the promising effect of a biosurfactant produced by *C. lipolytica* regarding the growth of autochthonous microorganisms in seawater and the enhancement of the biodegradation of motor oil at concentrations of ½ CMC, CMC and 2 × CMC over a 30-day period. The dispersant capacity of a biosurfactant is of extreme importance in the treatment of marine environments contaminated with hydrocarbons. This characteristic facilitates the access of autochthonous microorganisms to the pollutant, potentiating the bioremediation process (Luna *et al*., 2013). In the present study, the isolated crude biosurfactant from PA3 and PA4 promoted the accelerated growth of these microorganisms throughout the 30 days of culture and served as a solubilizing agent for motor engine oil, thereby facilitating its biodegradation.

Kinetic analysis is a key factor for understanding biodegradation process, bioremediation speed measurement and development of efficient clean up for a petroleum hydrocarbon contaminated environment. The information on the kinetics of soil bioremediation is of great importance because it characterizes the concentration of the contaminant remaining at any time and permit prediction of the level likely to be present at some future time. Petroleum hydrocarbon biodegradation rates are usually difficult to predict due to the complexity of the environment (Agarry and Oghenejoboh, 2015). A half-life of a biological biodegradation is the time taken for a substance to lose half of its amount and therefore, biodegradation half-lives are vital tools for many applications such as chemical screening, environmental modelling fate and description of pollutants transformation as specified by a number of investigators (Kachieng'a and Momba, 2017). In this study, the first-order kinetics model equation (Eqns 3, 4 and 5) fitted to the biodegradation data (Figures 5 and 6) was used to determine the rate of biodegradation oil contaminated soil and seawater treated through application of the consortium of yeast isolates and their biosurfactants, respectively. The values of the rate constants obtained from fitting of the model are presented in Table 3. The results in Table 4 as indicated by the high correlation determination (R^2) showed that the biodegradation of waste engine oil fitted well to the first-order kinetic model. The half-life time of waste engine oil biodegradation was calculated using eq. (6). The biodegradation rate constants (k) and half-life times ($T^{1/2}$) for the different remediation treatments are presented in Table 4. It is to be noted that the higher is the biodegradation rate constants, the higher or faster is the rate of biodegradation and consequently the lower is the half-life time. Table 4 showed that the biodegradation of waste engine oil in contaminated soil under Co (control) had a highest k value (0.0615 day^{-1}) and lower $T^{1/2}$ (11.5500 $day^{-1)}$ than that under C1 (consortium only) (0.0409 day^{-1} and $T^{1/2}$ = 15.0700 days), C2 (Consortium and 50 % biosurfactant) ((k = 0.0347 day^{-1} and $T^{1/2}$ = 13.0800 days) and C3 (Consortium and 100 % biosurfactant) (k = 0.0237 day^{-1} and $T^{1/2}$ = 12.8300 days), respectively. Also, the biodegradation of waste engine oil in contaminated seawater under C1 (consortium only) had a highest k value (0.0557 day^{-1}) and lower $T^{1/2}$ (10.6600 $day^{-1)}$ than that under Co (control) (0.0541 day^{-1} and $T^{1/2}$ = 11.3600 days), C2 (Consortium and 50 % biosurfactant) ((k = 0.0264 day^{-1} and $T^{1/2}$ = 13.0800 days) and C3 (Consortium and 100 % biosurfactant) (k = 0.0207 day^{-1} and $T^{1/2}$ = 11.3600 days), respectively Therefore, value of the kinetic parameter showed that the degree of effectiveness of these bioremediation strategies in the cleanup of soil contaminated with waste engine oil is in the following order: C3 < C2 < C1 < Co while the cleanup of seawater contaminated with waste engine oil is in the following order: Co < C3 < C2 < C1, respectively.

5. Conclusion

This study described the production of a low cost biosurfactant and demonstrated its applicability in the bioremediation of contaminated environments with waste engine oil. In the kinetic assays, the motor oil removal rate from soil was 97.4 % in a period of 60 days. In the tests performed with contaminated seawater, the oil removal rate was 85.8 % in 30 days. The biodegradation data for both assays were well fitted into pseudo first order kinetic model. The results demonstrated that the biosurfactant produced by yeast strains PA3 and PA4 had promising properties as a bioremediating agents for soil and water contaminated with waste engine oil and hydrocarbon compounds, respectively. Future study should focus on pilot or industrial scale production of biosurfactant with attendant cost implications.

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